# The *Arabidopsis* telomere sequence is highly abundant in the genome of *Phaseolus acutifolius* and preferentially located in the centromeres

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## Introduction

Telomeres are chromosomal structures aimed to protect the chromosome ends from degradation and fusion processes. In most organisms the telomeres consist of tandem repeats of a 4-10 bp sequence rich in Guanine. The first plant telomere sequence $(TTTAGGG)_n$ was isolated from *Arabidopsis thaliana* (Richards and Ausubel 1988). *In situ* hybridization and cloning showed that most plants investigated so far contain very similar telomeric motifs and that these motifs differ only in their copy number (e.g. Ganal et al. 1991, Schwarzacher and Heslop-Harrison 1991, Fuchs et al. 1995). In metaphase chromosomes of *Vigna unguiculata* (Galasso et al. 1995) and in polytene chromosomes of *Phaseolus coccineus* (Nagl 1991), the telomere sequence was found on almost all chromosomes in the terminal regions. Some studies, however, indicate that telomere sequences are not always located at the chromosome ends. They have also been observed at interstitial or centromeric regions (e.g. Richards et al. 1991, Fuchs et al. 1995, Gortner et al. in press). In this report we describe that the *Arabidopsis thaliana* telomere sequence $(TTTAGGG)_n$ shows a species-specific pattern in *P. acutifolius* in Southern hybridization, and that it is mainly located in the centromeric region of metaphase chromosomes.

## Material and methods

Southern hybridization of 20 *Dpn*II digested *Phaseolus* species including *P. vulgaris* (NI 573 and NI 1314), *P. coccineus* (NI 132), *P. acutifolius* var. *acutifolius* (NI 576), *P. acu.* var. *latifolius* (NI 562), and *P. acu.* var. *tenuifolius* (NI 692) was performed according to Hamann et al. (1995). As hybridization probe a synthetic oligonucleotide $(TTTAGGG)_5$, corresponding to the *Arabidopsis thaliana* telomere sequence (Richards and Ausubel 1988), was 3' end-labeled with digoxigenin-11-dUTP using an oligonucleotide kit (Boehringer Mannheim, BM). Blocking, prehybridization, and hybridization were carried out at 65°C as described by Hamann et al. (1995). Washing was performed once in 2 x SSC for 15 min at room temperature, twice in 2 x SSC for 15 min at 65°C and in 0.1 x SSC for 15 min at 65°C. Chemiluminescent signal detection was carried out with CDP-Star® (Tropix) according to Hamann et al. (1995).

For fluorescence in situ hybridization (FISH), chromosomes from root-tips of *P. acutifolius* accessions NI 576, NI 562, *P. coccineus* cv. Preisgewinner, and *P. vulgaris* cv. Hilds Marona were investigated. Metaphase chromosome spreads were prepared according to Schwarzacher et al. (1989) and *in situ* hybridization was carried out following the technique described by Gortner et al. (in press). As a probe, the same synthetic oligonucleotide was used as for Southern hybridization. Fluorescent hybridization sites were detected after one round of signal amplification according to Nenno et al. (1996) and documented with the help of a cooled CCD camera.

## Results and discussion

The *Arabidopsis thaliana* telomere sequence $(TTTAGGG)_n$ could be detected by Southern hybridization in the genome of all *Phaseolus* species analyzed. In all taxa, with the exception of *P. acutifolius* accessions, a typical hybridization pattern consisting in a strong smear in the high molecular weight regions above 6.5 kb was observed. Depending on the investigated species several faint but discrete bands (1-10), displaying different patterns, could be detected below 6.5 kb. This result is in accordance with other reports, where the oligomere $(TTTAGGG)_3$ produced a smear in the

In the *P. acutifolius* taxa NI 576, NI 562 and NI 692, however, the probe $(TTTAGGG)_5$ produced a species-specific hybridization pattern with many bands, completely covering the lanes on the X-ray film. Interestingly, the signal strength showed increasing intensity in the lower molecular weight range.

Fluorescence in situ hybridization (FISH) was carried in order to determine the chromosomal localization of the telomere repeat $(TTTAGGG)_5$ in the *P. acutifolius* accessions NI 576, NI 562, as well as in *P. vulgaris* and *P. coccineus*. On chromosomes of *P. coccineus*, FISH showed weak but distinct signals exclusively at the chromosome ends, what is in accordance with results in polytene chromosomes of *P. coccineus* (Nagl 1991). Hybridization sites in *P. vulgaris* displayed higher signal intensity and were located at the chromosome termini as well. In the two *P. acutifolius* accessions, however, strong signals were preferentially found in the centromere regions while only additional faint signals were visible at the ends of a few chromosomes.

The occurrence of the telomere sequence at the centromeres of *P. acutifolius* is quite remarkable. Guerra and Kenton (1996) studied the distribution of the telomere sequence in an amphidiploid *P. vulgaris* x *P. acutifolius* and found signals on most chromosome ends. Only a few chromosomes showed additional signals in the centromeric region and were regarded as "diffuse hybridization". In the light of our results, the observations on the amphidiploid of Guerra and Kenton (1996) might undergo a different interpretation: the chromosomes with telomeric signals are only those derived from the *P. vulgaris* progenitor, while those few chromosomes showing centromeric and telomeric hybridization sites are derived from *P. acutifolius*. These chromosomes either consist of *P. acutifolius* DNA only, or they are the result of intergenomic translocations between the parental genomes, similar to the observations in the amphidiploid *Nicotiana tabacum* (Kenton et al. 1993).

The stronger FISH signals of *P. acutifolius* as compared with those of *P. vulgaris* and *P. coccineus* correlate with the result from Southern hybridization, that the telomere repeat is most abundant in *P. acutifolius*. Furthermore, the two observations of increasing signal strength at decreasing molecular weight range and the strong FISH signals in the centromeric regions, give evidence for the assumption that the telomere sequence *P. acutifolius* is part of a centromeric satellite DNA.

The data presented here seem to provide a promising indication that the *Arabidopsis*-type telomere sequence might be applied as an useful molecular marker for *Phaseolus acutifolius* in breeding programs.

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